

CLAIMS

1. Biologically active recombinant DNA-derived factor IX protein having a specific activity defined as Clotting/activity Antigenic concentration as determined by ELISA of at least 90% of that of blood-derived factor IX, and free from contamination by poxvirus proteins.

05 2. Biologically active recombinant DNA-derived factor IX protein having a molecular weight of about 57 kilodaltons and free from contamination by precursor factor IX to an extent of more than 10 weight % and by poxvirus proteins.

10 3. Factor IX protein according to Claim 1 which is human factor IX protein or sufficiently similar thereto to be acceptable for infusion into human patients suffering from factor IX deficiency.

15 4. Factor IX protein according to Claim 2 which is human factor IX protein or sufficiently similar thereto to be acceptable for infusion into human patients suffering from factor IX deficiency.

20 5. A process of preparing factor IX protein as defined in Claim 1, which comprises preparing a recombinant expression vector, said vector not being of the poxvirus family, by linking a factor IX DNA sequence to a promotor sequence effective to express the DNA in a eukaryotic cell and incorporating these DNA sequences in a vector, and introducing the expression vector, in vitro, into eukaryotic cells having post-translational modifying means effective to modify the biologically inactive product of the expression of the DNA into the biologically active factor IX protein.

25 6. A process of preparing factor IX protein as defined in Claim 2, which comprises preparing a recombinant expression vector, said vector not being of the poxvirus family, by linking a factor IX DNA sequence to a promotor sequence effective to express the DNA in a eukaryotic cell and incorporating these DNA sequences in a vector, and introducing the expression vector, in vitro, into eukaryotic cells having post-translational modifying means effective to modify the biologically inactive product of the expression of the DNA into the biologically active factor IX protein.

7. A process according to Claim 5 wherein the factor IX DNA sequence comprises substantially all the cDNA sequence complementary to at least that part of factor IX mRNA which codes for the primary translation product.

05 8. A process according to Claim 6 wherein the factor IX DNA sequence comprises substantially all the cDNA sequence complementary to at least that part of factor IX mRNA which codes for the primary translation product.

9. A process according to Claim 7 wherein the DNA sequence 10 further comprises a non-coding sequence to the 5'-end of the coding sequence.

10. A process according to Claim 8 wherein the DNA sequence further comprises a non-coding sequence to the 5'-end of the coding sequence.

15 11. A process according to Claim 5, wherein the expression vector contains a gene providing a selectable marker for eukaryotic cells into which the vector has been introduced and the cells are selected with the aid of the marker.

12. A process according to Claim 5 wherein the eukaryotic cells 20 into which the expression vector is introduced are mammalian cells.

13. A process according to Claim 12 wherein the mammalian cells are liver or kidney cells.

14. A process according to Claim 5 wherein the vector is 25 introduced by transfection.

15. A process according to Claim 5 wherein the factor IX DNA is human factor IX DNA.

16. A process according to Claim 5 which further comprises recovering the biologically active factor IX protein from the 30 eukaryotic cells and purifying it by affinity chromatography.

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